CLAIMS

What is claimed is:

1. A method of making a reference library comprising a mixture of heterogeneous nucleic acid fragments, comprising:

digesting pooled nucleic acids comprising first restriction sites with a first restriction endonuclease to produce a mixture of restriction fragments;

forming a first population of single stranded nucleic DNA fragments from a first subpopulation of said restriction fragments, wherein said first subpopulation of restriction fragments comprises a second restriction site which is different from said first restriction site;

forming a second population of single stranded DNA fragments from a second subpopulation of said restriction fragments wherein said second subpopulation of said restriction fragments do not contain said second restriction site, and wherein said first single stranded DNA fragments have complementary sequences to said second single stranded DNA fragments from said second subpopulation when said single stranded DNA fragments are derived from the same restriction fragment;

hybridizing the first and second populations of single stranded DNA fragments to form a first population of duplexes;

treating said first population of duplexes with a single strand dependent nuclease to digest mismatched duplexes in said first population.

2. The method of Claim 1, further comprising:

reannealing intact single stranded DNA from said nuclease digestion to form a second population of duplexes; and

isolating said second population of duplexes to form a reference population of restriction fragments.

3. The method of Claim 1, further comprising the step of amplifying the matched duplexes using PCR.

4. The method of Claim 1, wherein said single strand dependent nuclease is selected from the group consisting of T7 endonuclease , S1 nuclease and mungbean nuclease.

5. The method of Claim 1, wherein said single strand dependent nuclease is T7 endonuclease.

6. The method of Claim 2, wherein said population of duplexes is isolated using biotin precipitation.

7. A method of making a reference library comprising a mixture of heterogeneous nucleic acid fragments, comprising:

digesting pooled nucleic acid comprising first restriction sites with a first restriction endonuclease to produce a mixture of first restriction fragments having first cleavage ends;

ligating an Exo III resistant linker to the first cleavage ends of said first restriction fragments to form a first ligation product;

digesting said first ligation product with a second restriction endonuclease to form a mixture of second restriction fragments, some of which comprise a second cleavage end;

ligating an Exo III susceptible linker to said second cleavage ends of said second restriction fragments to form a second ligation product population which includes said first ligation product,

wherein said Exo III susceptible linker comprises a first member of a binding pair;

digesting said second ligation product population with Exo III to form a third ligation product population comprising (i) single stranded DNA comprising end sequences corresponding to said Exo III resistant and Exo III susceptible linkers and (ii) double stranded DNA comprising end sequences corresponding to said Exo III resistant linkers;

denaturing said third ligation product population and hybridizing the mixture so obtained to form a reannealed third ligation product population; and

contacting said annealed third ligation product population with a second member of said binding pair to enrich for duplexes which form a reference population of restriction fragments.

8. The method of Claim 7, further comprising contacting said reannealed third ligation product with a single strand dependent nuclease.

9. The method of Claim 7, further comprising contacting said reannealed third ligation product population with exonuclease I.

10. The method of Claim 7, wherein said Exo III susceptible linker further comprises biotin.